Lehninger (1951, p. 7) concluded from these investigations that "mitochondria contain a complete complement of the individual enzymes of the fatty acid oxidase system and in such amount that they could easily account for the known rates of oxidation in the intact cell."[6] Lehninger and Kennedy also found that mitochondria contain all the needed enzymes for oxidizing intermediates of the citric acid cycle, although they could not rule out the possibility that other fractions might also contain some of these enzymes. They also made an important negative finding – the reactions of glycolysis were catalyzed by the supernatant, not the mitochondrial fraction or other fractions with insoluble cell components. They concluded that glycolysis occurred in the aqueous cytosol of the cell's cytoplasm. Its product, pyruvic acid, would therefore need to be transported into the mitochondrion to join fatty acid products in the common pathway of the citric acid cycle.

In further research carried out with another graduate student, Morris Friedkin, Lehninger established, using labeled $P^{32}$, that isolated mitochondria synthesized ATP (Friedkin & Lehninger, 1949). In these initial studies, though, the rate of ATP formation was very slow. Lehninger (1951) discovered that when DPNH (NADH) was added to the mitochondrial preparation, it did not penetrate the mitochondrial body. He found that DPNH could enter the mitochondrion if he placed the preparation into a hypotonic KCl, sucrose, or distilled water medium for a short period before restoring isotonicity. Under these circumstances, oxidative phosphorylation occurred robustly and, in accordance with what Ochoa had found for tissue extracts, producing approximately three ATP molecules per atom of oxygen consumed.

A distinctive feature of Lehninger's theoretical outlook was that he interpreted difficulties in developing biochemical preparations for studying oxidative phosphorylation as clues to the importance of mitochondrial structure for those processes. This applied not only to the difficulty in getting DPNH to enter the mitochondria, but also to the fact that oxidative metabolism could only occur in preparations in which cell particulates remained. For him the failure to extract the responsible enzymes for oxidative metabolism when

[6] Noting the need to supply metabolites such as malate (malic acid), $MgSO_4$, cytochrome *c*, KCl, and ATP to maintain the reaction, Kennedy and Lehninger concluded, "Although the mitochondria appear to be the major site of these activities, it would appear from our examination *in vitro* that these bodies are not completely autonomous with respect to their respiratory behavior, since they must be supplemented with certain cofactors such as adenosine triphosphate and Mg++. It appears likely that in the cell there is a rapid interchange of these factors, substrates, and inorganic phosphate between the cytoplasm and the mitochondria. It also would appear that these bodies are dependent on the cytoplasm for certain preparatory metabolic activities such as glycolysis, since, as our data show, they are almost completely lacking in glycolytic activity" (1949, pp. 970–1).